The Kell and Kx Blood Group Systems

Soohee Lee, Ph.D

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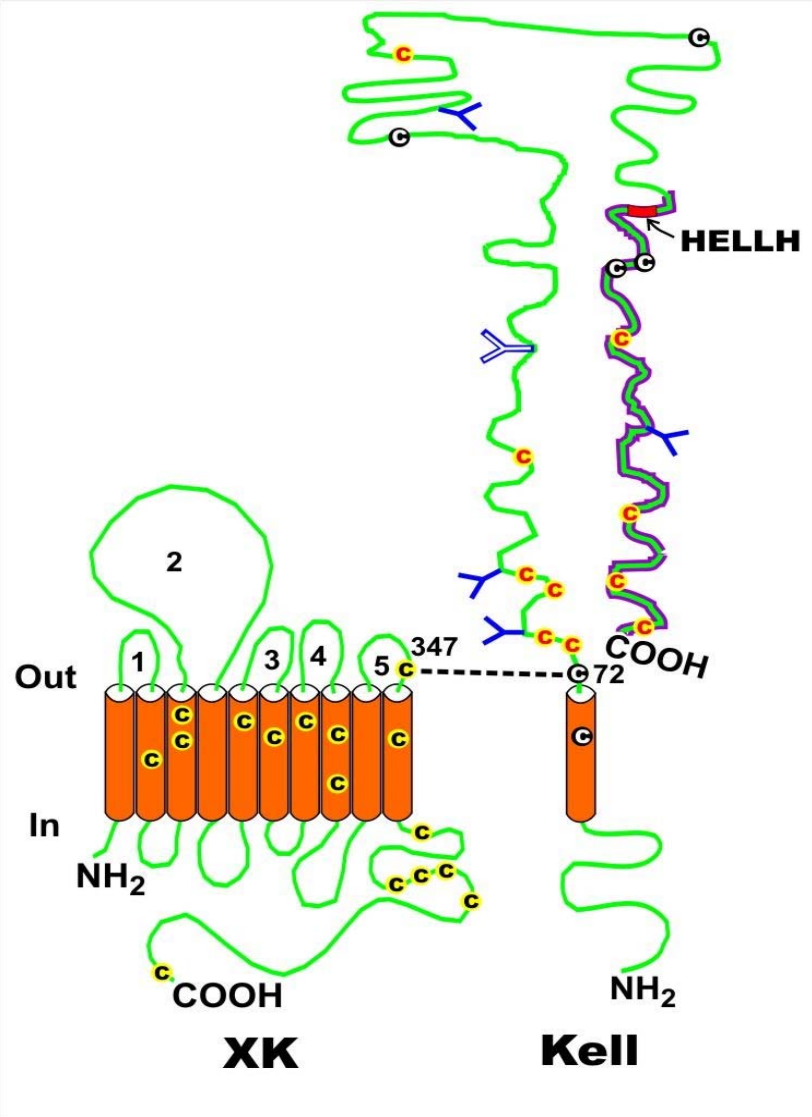
Kell Blood Group System

- **A major human blood group system that is highly polymorphic, expressing about 30 different alloantigens.**
- **Important in transfusion medicine after the ABO and Rh blood group systems since some of the antigens are potent immunogens.**
- **Kell antibodies can cause transfusion reactions in mismatched blood transfusions and fetal anemia in feto-maternal incompatible pregnancies.**

Kell and Kx Blood Group Systems

- **The Kell and Kx blood group systems are interrelated because Kell protein that carries Kell antigens and XK protein that carries a single antigen, Kx, are covalently linked on red cells through a single disulfide bond.**
- **Kell is a 93 kDa type II membrane glycoprotein having one TMR with a short intracellular N-terminal domain and a large extracellular C-terminal domain (Lee et al. *PNAS* 88:6353-6357, 1991)**
- **Kell is an endothelin-3-converting enzyme (Lee et al. *Blood* 94:1440-1450, 1999) and belongs to the M13 family of Zinc endopeptidases.**
- **XK is a 50.9 kDa, a putative membrane transport protein and traverses the membrane 10 times (Ho et al. *Cell* 77:869-880, 1994).**

Kell/XK Protein Complex



Y: N-linked Sugar residue

C: Conserved cysteine

C : Non-conserved cysteine

C : Cysteine in XK

HELLH: Zinc binding enzyme active site

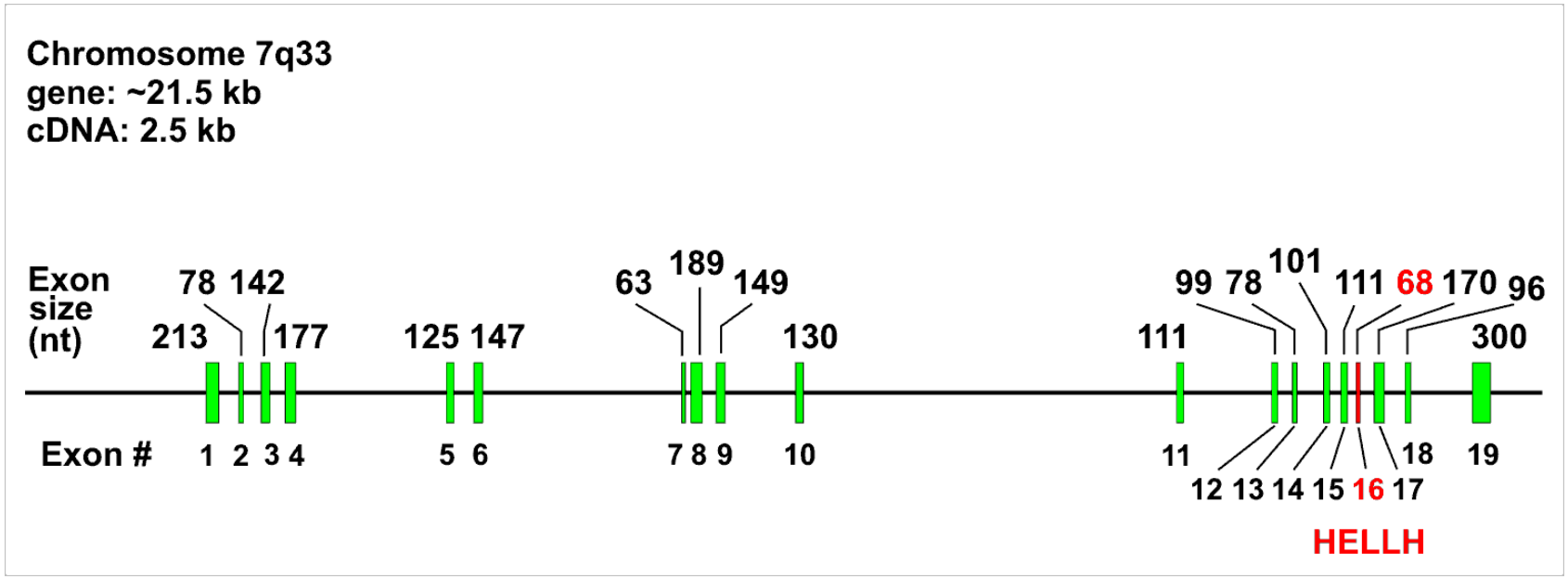
---------- : Disulfide linkage

Kell and XK on red cells

- **Two rare Kx and Kell blood group phenotypes have been instructive in defining the relation of Kell and XK proteins.**
- ***McLeod RBCs*: Lack Kx antigen and have very weak expression of all Kell antigens. Western blots show lack of XK and diminished amount of Kell glycoprotein.**
- ***Kell(null) RBCs*: Lack all Kell antigens but have enhanced Kx antigen. However, Western blots show diminished amount of XK protein. Probably, in the wild-type, Kell protein partially covers Kx antigen.**

Organization of Human Kell Gene (*KEL*)

Lee et al. *Blood* 85:1364-1370,1995



HELLH: Zinc binding enzyme catalytic motif

Molecular Basis of Kell Antigens

Antigen (wild type/variant)	Exons	Nucleotide Change (wild-type to variant)	Amino Acid Change (wild-type to variant)	Restriction Enzyme change (Wild-type to variant)
KEL2(k)/KEL1(K)	6	698C>T	T193M	*Bsm*I+
KEL4 (Kp^b)/KEL3 (Kp^a)	8	961C>T	R281W	*Nla*III+
KEL4 (Kp^b)/KEL21 (Kp^c)	8	962G>A	R281Q	PvuII+
KEL7 (Js^b)/KEL6 (Js^a)	17	1910T>C	L597P	*Mnl*I–
	17	2019A>G	L633(Silent)	*Dde*I–
KEL11/KEL17(Wk^a)	8	1025T>C	V302A	*Hae*III+
KEL10–(Ul^a–)/KEL10+(Ul^a+)	13	1601A>T	E494V	*Acc*I+
KEL12+/KEL12 –	15	1763A>G	H548R	*Nla*III
KEL13+/KEL13–	9	1106T>C	L329P	
KEL14/KEL24	6	659G>C	R180P	*Hae*III+
KEL18+/KEL18–	4	508C>T	R130W	*Taq*II+
	4	509G>A	R130Q	*Eco57*I+
KEL19+/KEL19–	13	1595G>A	R492Q	
KEL22+/KEL22–	9	1085C>T	A322V	*Tsp45*I+
KEL23–/KEL23+	10	1265A>G	Q382R	*Bcn*I+
KEL25– (VLAN–)/KEL25+ (VLAN+)	8	863G>A	R248Q	*PspG*I+*
KEL26+(TOU+)/KEL26 –(TOU–)	11	1337G>A	R406Q	
KEL27+(RAZ+)/KEL27–(RAZ–)	8	865G>A	E249K	*EcoRI*+*
KEL28–(VONG–)/KEL28+(VONG+)	8	862C>T	R248W	
KEL29+(KALT+)/KEL29–(KALT–)	17	1988G>A	R623K	*TfiI*–*
KEL30+(KTIM+)/KEL30–(KTIM–)	8	1033G>A	D305N	*Taq*I–*
KYO–/KYO+	8	995G>A	R292Q	

*Mutation(s) introduced into primer(s)

KEL1(K, K1) and KEL6 (Js^a, K6) Phenotypes

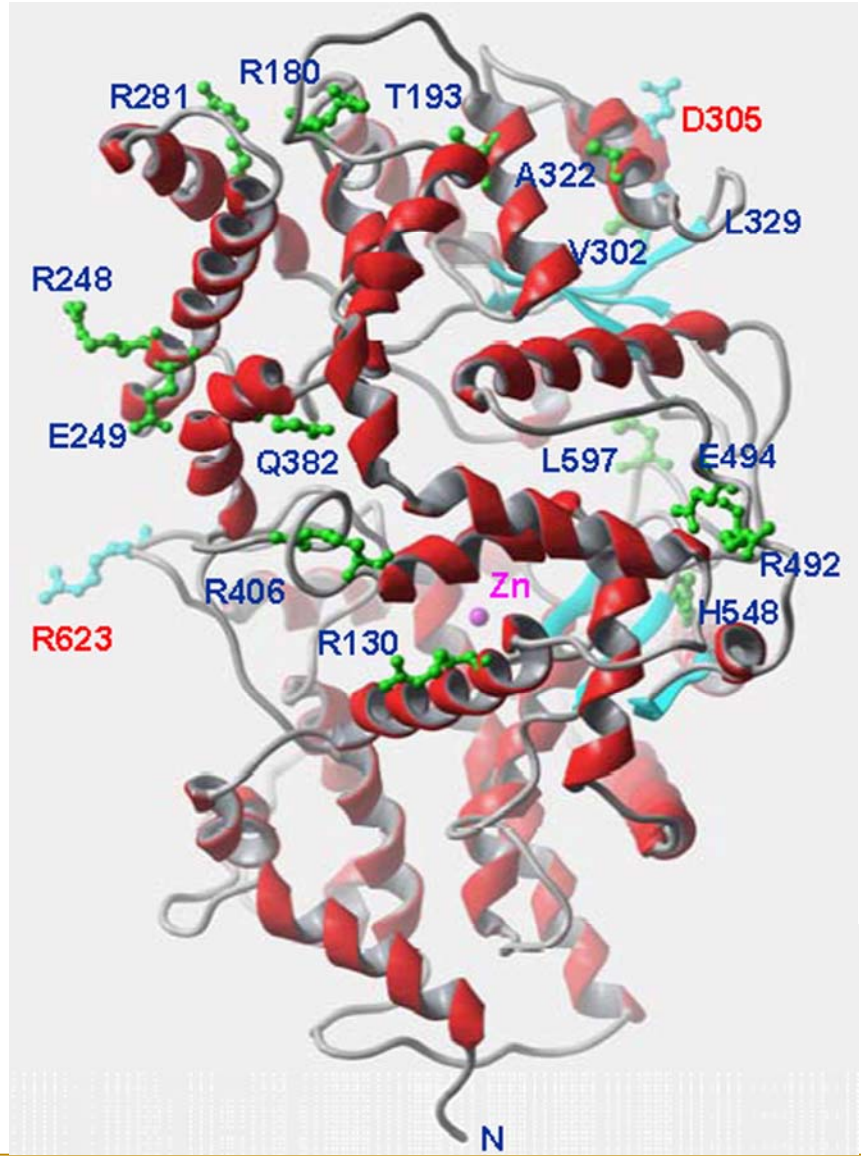
KEL1 (K, K1):

- **Antithetical with KEL2 (k, K2)**
- **9% in Caucasians, 2% in Blacks**
- **698C>T (exon 6), T193M**
- **One N-linked sugar residue is removed.**

KEL6 (Js^a ,K6):

- **Antithetical with KEL7 (Js^b ,K7)**
- **19.5% in Blacks, <1% in Caucasians**
- **1910T>C (exon 17), L597P**
- **The mutation is located near the Zn-binding enzyme active site between two cysteines at 596 and 599.**

Locations of the amino acid residues responsible for Kell polymorphisms



Lee et al. *Transfusion*, 46:1323-1327, 2006

KEL$_{null}$ (K_o) Genotypes

Location	Nucleotide Mutation	Mutation	Zygosity
Taiwan	IVS3+1G>C	Alternative Splicing	Homozygous
Afro-American, USA (2)	502C>T, exon 4	R128Stop	Homozygous
Yugoslavia	366T>A, exon 4	C83Stop	Homozygous
Portugal	1162C>T, exon 9	Q348Stop	Homozygous
Israel	2147G>A, exon 18	S676N	Homozygous
Reunion Island & Linkoping, Sweden	IVS3+1G>A	Alternative Splicing	Homozygous
Seattle	1208G>A, exon 10 IVS3+1G>A	S363N* Alternative Splicing	Heterozygous
New York	1208G>A, exon 10 694C>T, exon 6	S363N* R192Stop	Heterozygous
Japan	1497G>A, Exon12 IVS5–2A>G	W459Stop Alternative Splicing	Heterozygous
Uppsala, Sweden	1540C>T, Exon 13	Q474Stop	Homozygous
Umea, Sweden	1023 delG, Exon 8	Frame shift	Homozygous
Holland	IVS7-1G>C	Alternative Splicing	Heterogygous**
Holland	IVS8+1G>T	Alernative Splicing	Heterogygous**
Germany	1666C>T	R516Stop	Homogygous

*Small amount of Kell protein identified in homozygotes (KEL_{mod}).
**The other allele is *KEL*1*

KEL_{mod} (K_{mod}) phenotype:

- **K_{mod} is an inherited rare RBC phenotype characterized by weak but detectable expression of high-incidence Kell antigens.**
- **Different point mutations cause amino acid substitutions that may alter protein conformation inhibiting transport of the mutant Kell proteins to the cell surface.**
- **The depression of the antigen is intensified when paired with a *KEL_{null}* allele.**

Point Mutations in 4 Unrelated Individuals with K_{mod} Phenotype

Phenotype	Nucleotide mutation	Amino Acid substitution	Exon	Zygosity
K_{mod}–1	1208G>A	S363N*	10	Homozygous
K_{mod}–2	1208G>A 2150A>G	S363N* Y677C	10 18	Heterozygous
K_{mod}–3	1106T>C 1716G>A	L329P (KEL13) W532Stop (Null)	9 15	Heterozygous
K_{mod}–4	2227G>A 1839C>T	G703R Silent	19 16	Heterozygous KELnull allele

*Sometimes typed as KEL_{null} when paired with a *KEL_{null}* allele

KEL1 Sensitization in Pregnancy, Survey 1

- **Anti-KEL1 (anti-K) is the second most common antibody after anti-D causing complications in pregnancies and is produced mostly by incompatible blood transfusions and in some cases by previous pregnancies. Anti-KEL1 currently accounts for about 10% of the cases of severe anemia of newborns.**
- **In 127,076 pregnancies, 127 had antibodies to KEL1.**
- **13 of them had KEL1 positive babies.**
- **5 had serious perinatal complications (hydrops and neonatal death)**

(Caine et al. *Am. J Obstet. Gynecol*. 154:85-90, 1986)

Maternal KEL1 Alloimmunization, Survey 2

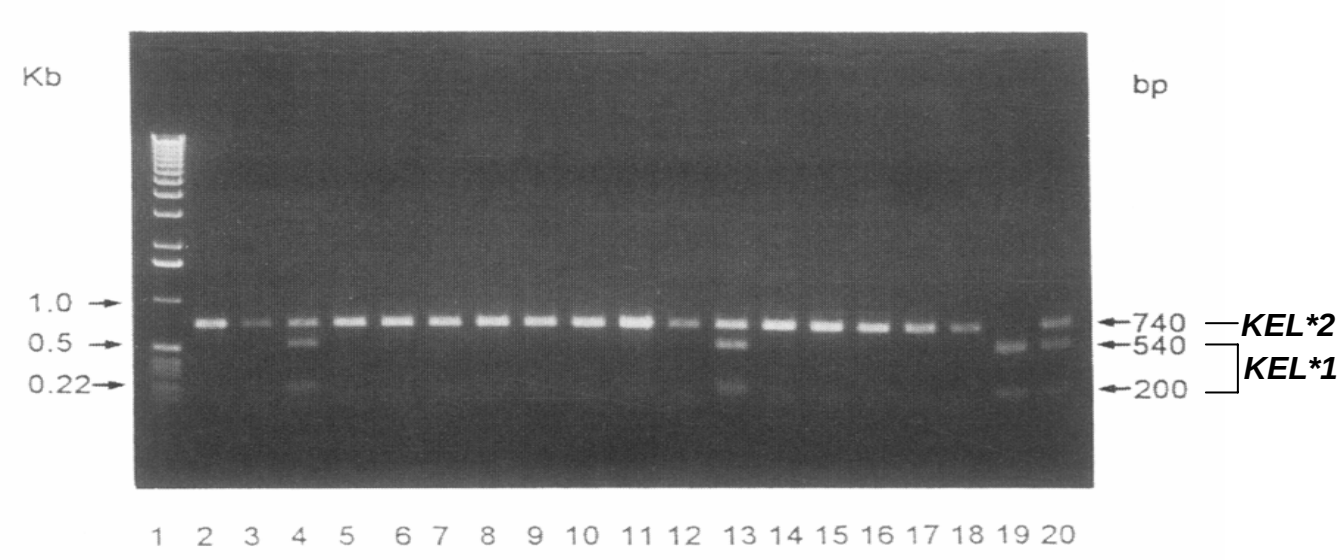
- **Between 1944 to 1990, 311 Manitoban women were KEL1 (K) immunized and had 459 pregnancies.**
- **63 ended in abortion or stillbirth unrelated to anti-KEL1.**
- **376 were unaffected.**
- **20 were affected (4.4%), of which 12 did not require treatment, 4 received treatment and 4 resulted in death.**

  (Bowman et al, *Obstet Gynecol*, 1992, 79:239-244)

Causes of Fetal Anemia Related to Anti-KEL1

- **Fetal anemia related to anti-Kell antibodies is thought to be due to the inhibition of erythropoiesis rather than hemolysis of red cells (**Vaughan et al. *New Engl. J Med*. 338:798-803, 1998**).**
- **Kell glycoprotein is expressed in early erythoid progenitor cells** (Southcott et al. *Blood* 93:4425-35, 1999), **and Anti-KEL1 related fetal anemia is caused by promoting the immune destruction of KEL1 positive cells, by macrophages, at an early progenitor cell stage** (Daniels et al. *Transfusion* 43:115-116, 2003).
- **Therefore, unlike RhD sensitization, anti-KEL1 titers and bilirubin levels of maternal blood are not good predictors of fetal anemia. Fetal *KEL* genotyping is required.**
- **When a mother is KEL1 negative, the father should be typed to determine the potential for a *KEL*1* baby. If the father is KEL:1,2 phenotype, fetal genotyping should be performed to determine if further interventions are necessary. Procedures for fetal genotyping have been authenticated** (Lee et al. *Am. J. Obstet. Gyneco.* 175:455, 1996).

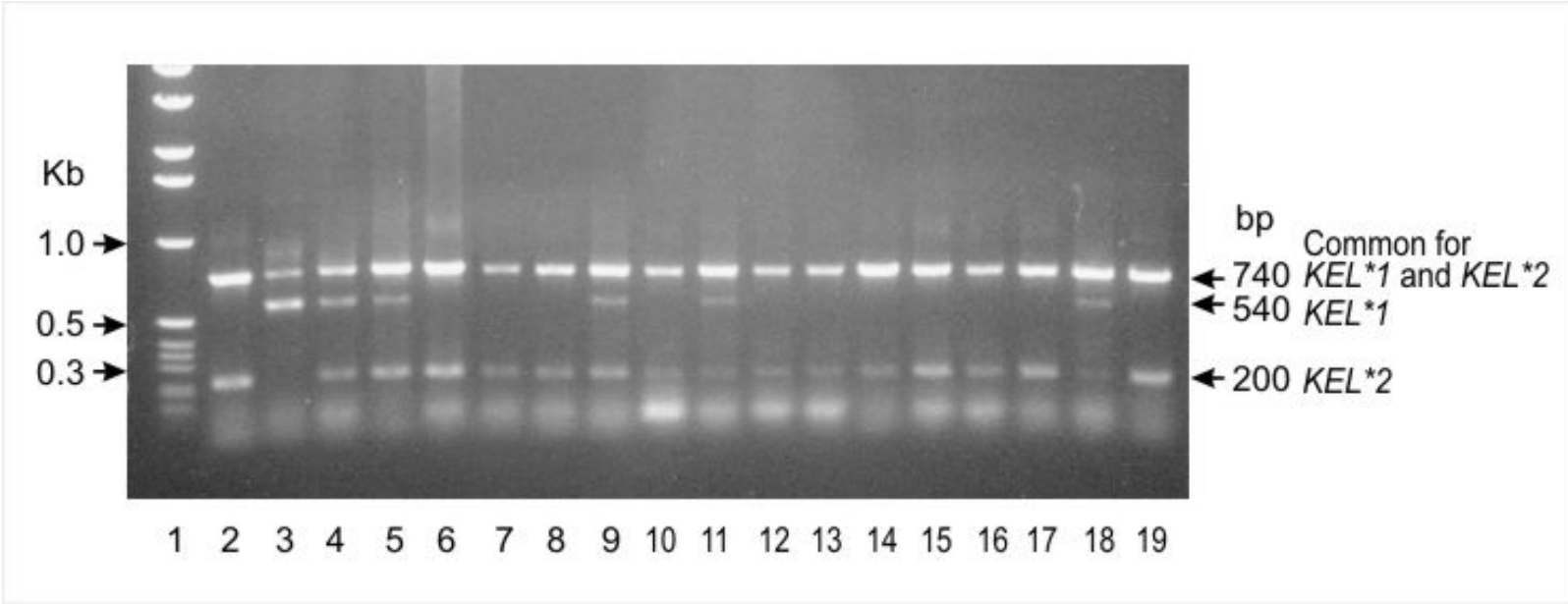
Prenatal Diagnostic Genotyping of *KEL1//2* (*K/k*) by *Bsm*I RFLP



Lane 1, DNA ladder;
Lane 2, PCR Product from peripheral blood untreated with BsmI
Lanes 18 (*KEL2/2*), 19 (*KEL*1/1*) and 20 (*KEL1/2*) , control DNA from peripheral blood,
Lanes 5 to 12 and 14 to 17, *KEL*2/2,* amniotic fluid DNA sample
Lanes 4 and 13, *KEL*1/2*, amniotic fluid DNA sample

Lee et al., *Am.J.Obstet.Gynecol.* 175(2):455-459, 1996.

*KEL*1/2* Genotyping by Allele-Specific PCR



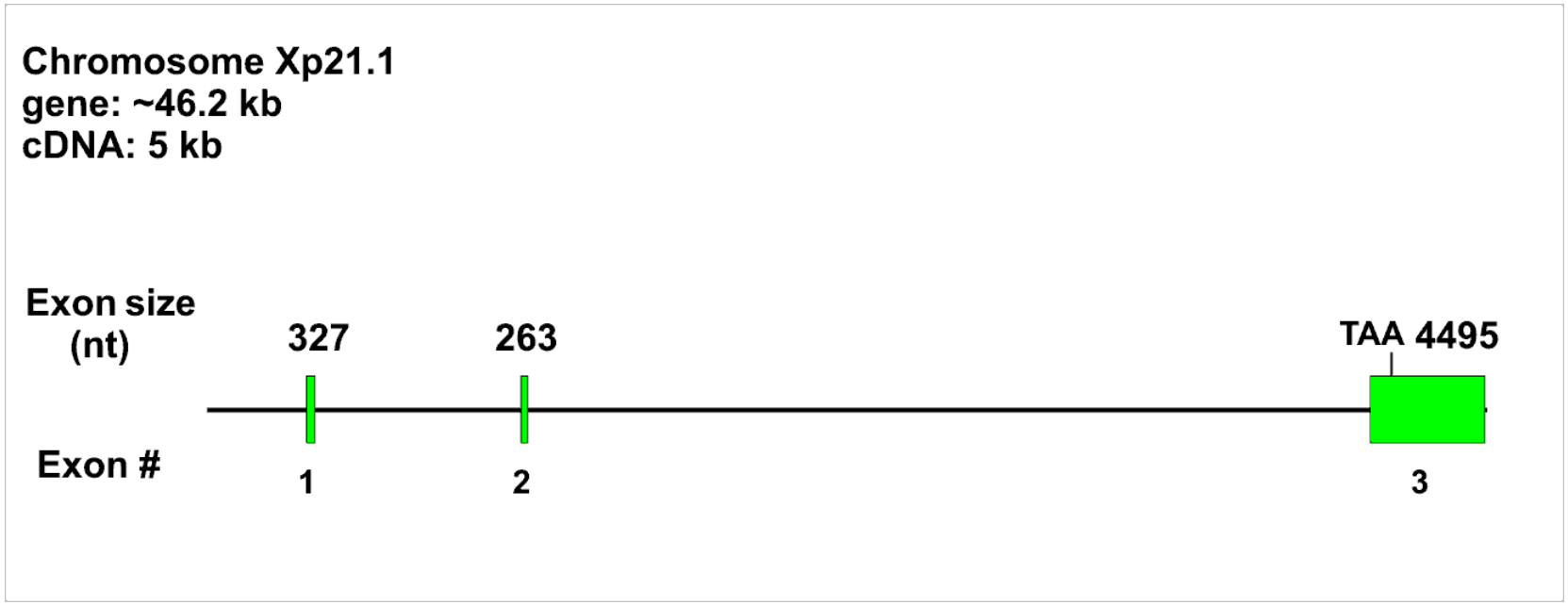
Lane 1, DNA ladder
Lanes 2, 6, and 7 (*KEL*2/2*), DNA from peripheral blood
Lanes 4, 5 and 9 (*KEL*1/2*), DNA from peripheral blood
Lanes 3 (*KEL1/1*), DNA from peripheral blood with KEL:1,-2 phenotype
Lanes 8, and 10 to 19, amniotic fluid DNA samples, lanes 11, and 18, *KEL*1/2*

Lee et al., *Am.J.Obstet.Gynecol.* 175(2):455-459, 1996.

Kx Blood Group System

- **Composed of a single antigen Kx.**
- **The function of XK protein is not known but absence of XK, found in the McLeod phenotype, is associated with red cell acanthocytosis and late onset forms of neuromuscular abnormalities known as the McLeod syndrome.**
- **McLeod phenotype is identified by serological testing by absence of Kx antigen and weak expression of all Kell antigens but ultimate confirmation is by XK gene genotyping.**

Organization of Human XK Gene (*XK*)



Compilation of McLeod genotypes

- Gene deletion (8): absence of XK
- 1 bp deletion (4): premature stop codon
- 2-14 bp deletion (4): Aberrant protein
- Splice junction mutation (4): Aberrant protein
- Near splice junction mutation (1): Possible low level of normal XK
- 1 bp Insertion (1): Aberrant protein
- Point mutation resulting in stop codon (6): Truncated XK
  - 189G>A, W36Stop
  - 479C>T, R133Stop (5 unrelated cases)
  - 545C>T, Q155Stop
  - 789G>A, W236Stop
  - 997C>T, Q299Stop
  - 1023G>A, W314Stop
- Missense mutation (3): Mutant XK.
  - 746C>G, R222G (6^{th} TMR): absence of surface mutant XK
  - 962T>C, C294R (8^{th} TMR): absence of Kx antigen
  - 1061G>A, E327K (9^{th} TMR): absence of Kx antigen

Summary

- **The Kell blood group system is the most important system after the ABO and Rh systems because it is highly polymorphic and some of the antigens are strong immunogens.**
- **There are about 30 known antigens and all result from single nucleotide mutations that change single amino acids. Genotyping for the Kell antigens is possible by utilizing RFLP or allele-specific PCR methods.**
- **Among Kell antigens, KEL1 is the most immunogenic and can cause transfusion reactions in mismatched transfusions and fetal anemia in feto-maternal incompatible pregnancies.**
- **Kell and Kx blood group systems are interrelated because the Kell protein, that carries Kell antigens and the XK protein, that carries a single antigen, Kx, are covalently linked on red cells through a single disulfide bond.**
- **By serology, absence of the Kx antigen and weakened expression of Kell antigens identify the McLeod phenotype that manifests red cell acanthocytosis and late onset forms of neuromuscular abnormalities known as the McLeod syndrome.**
- **McLeod genotypes result from various gene mutations. Genotyping requires sequencing the PCR products of the exons and the exon-intron junctions of the *XK* gene.**